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Screening of Garden Pea Genotypes for High Temperature Tolerance Using Temperature Induction Response Technique

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A B S T R A C T

Garden pea is a cool season leguminous vegetable crop with a temperature requirement of 15-23°C, for its optimum growth. Thus, it is mainly grown as a *rabi* season crop in India. Increased CO_2 concentration in the environment has led to global warming. All the seedlings of pea lines were taken in uniformly after 48h and grown in completely randomized design (CRD) block with three replications having twenty seedlings per replication. The experiment was conducted in the laboratory of Division of Plant Physiology and Biochemistry, ICAR- Indian Institute of Horticultural Research, Bengaluru. The standardization studies using 48 h old Azad P-1 seedlings revealed that optimum lethal and induction temperature were 43°C for 3 hours and 30-38°C for 3 hours, respectively. Screening of 32 garden pea germplasm lines using TIR technique resulted in identification of 10 tolerant lines (ArkaUttam, Arka Apoorva, IIHR 544, IIHR 13-1, IIHR 680, PMR 37, Swarna Mukti, KTP 4, and VRPMR 11) with the help of normal Z-distribution.

Introduction

Garden pea botanically known as *Pisumsativum* var. *Hortense*, which belongs to family *Fabaceae*. It is cool season leguminous vegetable crop which is mainly grown as a *rabi* season crop in India. It is extensively grown for seeds as well as pods and it can be consumed as fresh, canned, frozen or dry seeds in different areas of the world. The optimum temperature reported for the cultivation of garden pea ranges from 15°C to 23°C with a maximum temperature of 28°C (Lambert and Linck, 1958; Nonnecke *et al.*, 1971).

They have been pretty well known for their nutritional values. Peas are an excellent source of protein, fibre, minerals and vitamins. The protein content of wrinkled-seeded pea cultivars is 26-33 % while the protein in their smooth seeded counterparts is 23-31%

(Cousin, 1997). Pea seed is a source of vitamins A, B, C and contains 35-40% starch, 4-7% fibre and relatively high levels of lysine. This makes it an appropriate dietary complement to cereals. In addition to garden pea's ability to fix atmospheric nitrogen, they improve soil structure, and provide breaks for disease control, which ensures their importance in modern agricultural systems (McPhee, 2004; Martin *et al.,* 2008).

Heat stress is defined as the rise in temperature above the optimum level for a period of time sufficient to cause irreversible damage to plant growth and development. It is a complex function of intensity, duration of exposure and rate of increase (Wahid *et al.,* 2007). Heat stress is a serious threat to crop production worldwide (Hall, 2001). It has been found to be associated with decrease in primary root growth and distribution of laterals (Gladish and Rost, 1993). High temperature stress causes various morphological, physiological, and biochemical changes in plants. Hence, the present investigation is carried out to identify garden pea genotypes for high temperature tolerance using temperature induction response.

Materials and Methods

Germplasm lines

Thirty-two garden pea germplasm lines available with ICAR- Indian Institute of Horticultural Research, Bengaluru were screened for tolerance to high temperature. The one-year old seeds of all the lines were collected from division of vegetable crops. All the lines were listed in the Table 1.

Experimental details

The experiment was conducted in the laboratory of division of plant physiology and biochemistry, ICAR- Indian Institute of Horticultural Research, Bengaluru. As, the whole experiment was conducted in controlled environment, the temperature at which seeds were allowed to germinate as well as recover, after their exposure to high temperature, was 25˚C (i.e. room temperature).

The seedlings, which were kept on water-soaked germination sheets in covered aluminium trays of 30x3x1cm^3 dimension, were exposed to high temperature stress in controlled growth chamber using a novel technique of Temperature Induction Response to screen genotypes for cellular level thermotolerance. All the seedlings of pea lines were taken in uniformly after 48h and grown in completely randomized design (CRD) block with three replications having twenty seedlings per replication.

It was analyzed in ANOVA (Panse and Sukhatme, 1978) followed by z distribution to observed the genotypes for high temperature tolerance. Z- Distribution, based on z-score of per cent seedling survival and per cent reduction in growth, was employed to group the genotypes in accordance to their response to high temperature using the following formula:

$$z\ score\ for\ \%\ seedling\ survival\ for\ variety\ A = \frac{Mean\ for\ var.A - Over\ all\ mean}{Standard\ deviation}$$

The observation was recorded by using following formulas;

$$Percent seedling survival\ (\%) = \frac{number of seedlings survived}{number of seedlings kept} \times 100$$

$$Recovery growth of seedlings\ (cm) = \frac{total growth of all the seedlings survived}{total number of seedlings kept}$$

$$Absolute control growth\ (cm) = \frac{total growth of seedlings kept as control}{total number of seedlings kept as control}$$

$$Percent\ reduction in growth = \frac{absolute control growth - recovery growth of seedlings}{(absolute control growth)} \times 100$$

Standardization of lethal temperature

Lethal temperature is the temperature at which seedling survival was lesser than 15 per cent. To standardize the lethal temperature (43˚C) as well as its duration (3 hours), young and uniform seedlings were exposed to different temperatures for different durations in controlled growth chamber. After their exposure to high temperature, the seedlings were allowed to recover at room temperature for 72 hours. At the end of the recovery period, the per cent survival of seedlings was worked out. The temperature at which seedling survival per cent was around 15per cent was fixed as lethal temperature for the experiment.

Standardization of induction temperature

Induction temperature is a sub lethal temperature at which when the seedlings are exposed, prior to lethal temperature, the maximum recovery growth was observed. To standardize the induction temperature as well as its duration, 48hour old seedlings of Azad P-1 were exposed to different temperatures for different durations, followed by lethal temperature. At the end of the recovery period the seedlings were observed for recovery growth, the set of treatment which showed maximum recovery growth was taken as optimum induction temperature.

Results and Discussion

Standardization of lethal temperature

The lethal temperature has been standardized by exposing two days old Azad P-1 seedlings to 48°C-3 hour, 45°C-3 hour, 44°C-3 hour, 43°C-3 hour and 42°C-3 hour (Table 2). The results as shown in the table below depicts only 10% seedling survival in 43°C-3 hour, whereas, at higher temperatures not a single seedling survived, but in case of the high temperature treatment where seedlings were exposed to 42°C-3 hour, all the seedlings survived thus, from these results it was concluded that 43°C-3 hour is standard lethal temperature for Azad P-1 seedlings. The percent reduction in growth over the absolute control, which can be used as other criteria for lethal temperature standardization along with per cent seedling survival, was 96.47% in this treatment.

High temperature response varies from crop to crop even from cultivar to cultivar, thus, standardization of lethal temperature is an important step prior to screening of germplasm lines in any given crop. In order to standardize lethal temperature, the seedlings should be exposed to different regimes of high temperature to look out for a high temperature at which seedling mortality is very high, but not 100 per cent. The standard definition for lethal temperature followed during the experiment goes as follows: the temperature at which the seedling survival per cent is between 10 and 15 % (Srikanthbabu *et al.*, 2002).

Standardization of duration of lethal temperature

After fixation of lethal temperature, identifying its duration is the next step, as temperature stress is a function of both intensity as well as duration. Thus, for standardization of duration of lethal temperature two days old Azad P-1 seedling were exposed to different durations (3 hours, 2hours 30 minutes and 2 hours) of lethal temperature *i.e.*, 43°C. The results obtained from this experiment have been presented in Table 3, where it shows that only 11.67% seedlings survived the after a recovery period of 72 hours. As, per cent seedling survival is an important criterion for fixation of lethal temperature and its duration, thus, 43°C for a duration of 3 hours was considered to be the

optimum lethal temperature. The percent reduction in growth over the absolute control was 90.04% which ensures that the duration for the lethal temperature is ideal for the experiment. Significant difference has been observed between the treatments for per cent seedling survival/mortality.

Standardization of induction temperature

Induction temperature is a pre-lethal temperature treatment, which instead of killing the seedlings, induce the cellular mechanism to in the seedlings to tolerate lethal temperature. In other words, the seedlings exposed to induction temperature prior to lethal temperature has more chances of survival after the recovery phase of 72 hours and tend to have a good recovery growth. Thus, in order to standardize induction temperature in two days old Azad P-1 seedlings, they were exposed to different induction temperature treatments (30-38°C-3 hour, 30-40°C-3 hour and 30-40°C- 2 hour) followed by exposure to the defined lethal temperature *i.e.*, 43°C for 3 hour (Table 3). For the treatments a gradual increment of temperature was maintained at a rate of 2.6°C/hour, 3.3°C/hour and 5°C/hour. All the seedlings belonging to different treatments were then allowed to recover at 25°C (room temperature) and data for % seedling survived, recovery growth of seedlings, absolute control growth and per cent reduction in growth was recorded. A non-induced control was maintained at 25 °C (room temperature). The maximum recovery growth (2.50) and per cent seedling survived (95.00%) have been reported in 30-38°C (3h), due to which it was considered as standard induction temperature.

After the standardization of lethal temperature and its duration, the next important step to be followed is to standardize the gradual induction temperature protocol. An induction temperature is a semi-lethal temperature at which, the exposed seedlings induce cellular tolerance to the lethal temperature and thus, the recovery growth of such seedlings is enhanced as compared to their exposure to lethal temperature directly. For this experiment the standard definition used is as follows: the temperature at which recovery growth of seedlings is maximum after a recovery period of 72 hours (Srikanthbabu *et al.*, 2002).

Table.1 List of garden pea germplasm lines used for screening for high temperature tolerance

Sl. No.	Genotype	Sl. No.	Genotype
1.	Arka Priya	17.	IIHR 680
2.	ArkaAjit	18.	IIHR 698
3.	Arka Pramod	19.	NDVP 24
4.	ArkaUttam	20.	PMR 37
5.	ArkaSampoorna	21.	PMR 53
6.	Arka Apoorva	22.	Swarna Mukti
7.	Arka Tapas	23.	KTP-4
8.	Arka Chaitra	24.	PC-531
9.	Arka Karthik	25.	Kashmir 13-1
10.	IIHR 1-2	26.	VRPMR-10
11.	IIHR 13-1	27.	VRPMR-11
12.	IIHR 13-15	28.	Azad P-1
13.	IIHR 13-18	29.	PM Var-7
14.	IIHR 13-22	30.	Oregon Sugar
15.	IIHR 13-29	31.	VRP 22
16.	IIHR 544	32.	PB 29614 X 7-6

Table.2 Standardization of lethal temperature for 48 h old Azad P-1 seedlings

Temp °C	Duration (hours)	% seedling survived after 72 h	Recovery growth of seedlings	Absolute Control growth	Per cent reduction in growth
48.0	3.0	0.00 (0.00)	0.00	7.22	100.00 (90.00)
45.0	3.0	0.00 (0.00)	0.00	6.45	100.00 (90.00)
44.0	3.0	0.00 (0.00)	0.00	7.32	100.00 (90.00)
43.0	3.0	10.00 (18.04)	0.26	7.45	96.47 (79.19)
42.0	3.0	100.00 (90.00)	4.42	8.06	45.12 (42.13)
Mean ± SEm		22.00 (21.61±1.28)	0.94±0.21	7.3	88.32 (78.27±1.57)
CD at 1%		5.72	0.95	-	7.05
CV (%)		10.23	39.15	-	3.48

Table.3 Standardization of duration of lethal temperature for 48 h old Azad P- 1 seedlings

Temp °C	Duration (hours)	% seedling survived after 72 h	Recovery growth of seedlings	Absolute Control growth	Per cent reduction in growth
43.0	3.0	11.67 (19.30)	0.64	6.39	90.04 (72.76)
43.0	2.5	41.67 (40.15)	1.66	7.22	76.96 (61.50)
43.0	2.0	93.33 (78.08)	1.39	5.17	73.05 (58.79)
Mean ± SEm		48.89 (45.84 ± 4.69)	1.23 ± 0.27	6.26	80.02 (64.35 ± 3.57)
CD at 1%		24.57	1.44	-	18.72
CV (%)		17.71	38.50	-	9.61

Table.4 Standardization of induction temperature followed by exposure to lethal temperature of 43 °C for 3 hours using 48 h old seedlings of Azad P-1

Induction Temp (°C) and Duration (hours)	% seedling survived after 72 h	Recovery growth of seedlings	Absolute Control growth	Per cent reduction in growth
30-38°C (3 h)	95.00 (79.53)	2.50	7.97	68.59 (55.98)
30-40°C (3 h)	43.33 (40.95)	1.79	7.97	77.57 (61.74)
30-40°C (2 h)	65.00 (54.82)	2.00	7.97	74.85 (60.01)
Mean ± SEm	67.78(58.43 ± 7.16)	2.10 ± 0.28	7.97	73.67 (59.24 ± 2.22)
CD at 1%	37.51	1.44	-	11.62
CV (%)	21.21	22.73	-	6.48

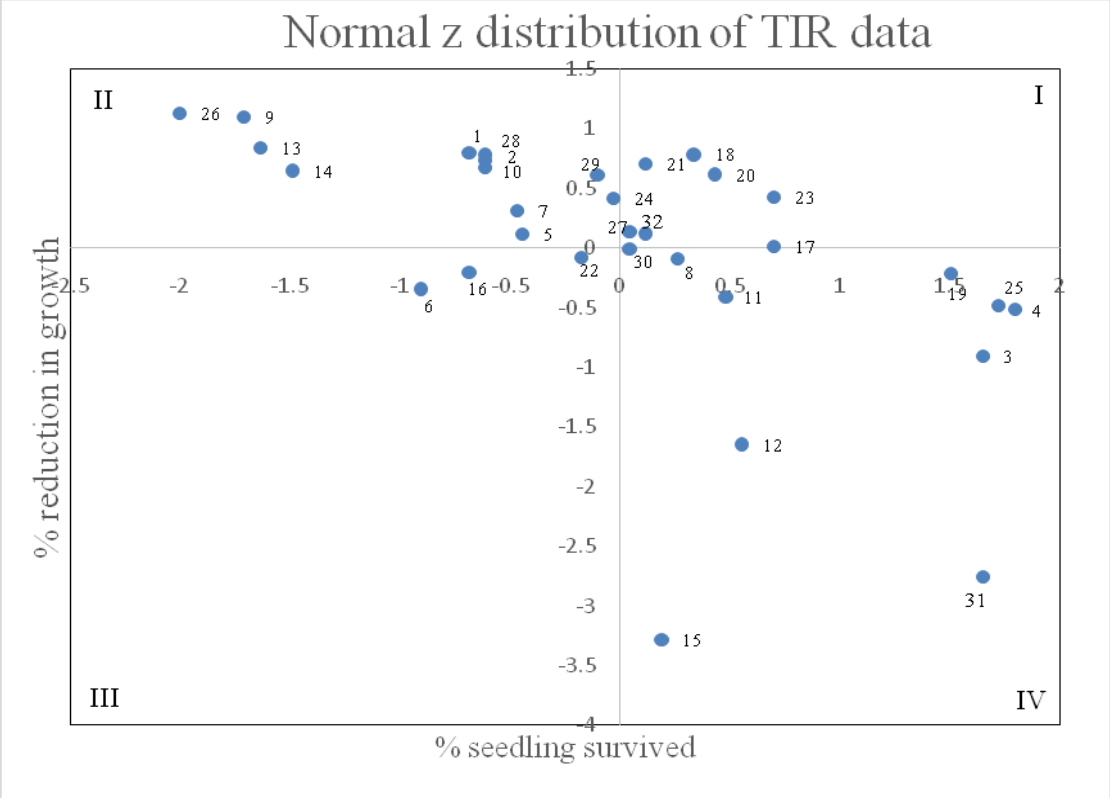
Table.5 Screening of 32 garden pea germplasm lines for high temperature tolerance at seedling stage

Lines	Per cent seedlings survived	Recovery growth of seedlings	Absolute Control growth	Per cent reduction in growth
IIHR 698	43.33 (40.62)	0.98	16.69	94.13 (77.68)
Kashmir 13-1	45.00 (42.07)	0.67	9.89	93.24 (76.60)
IIHR 13-1	96.67 (83.85)	3.77	12.07	68.75 (56.02)
IIHR 680	100.00 (90.00)	3.60	14.15	74.56 (59.77)
IIHR 13-22	48.89 (44.78)	1.40	8.74	83.98 (68.80)
Arka Pramod	38.33 (37.58)	1.13	4.94	77.14 (66.41)
NDVP 24	48.33 (44.03)	1.20	9.19	86.94 (69.63)
VRPMR 11	65.00 (54.02)	1.49	7.81	80.87 (64.93)
PB 29614 X 7-6	20.00 (26.44)	0.11	7.95	98.60 (83.24)
9/2014 PMVAR 7	45.00 (41.94)	0.57	7.42	92.34 (74.13)
ArkaUttam	70.00 (63.28)	1.37	5.75	76.14 (63.57)
KTP 4	71.67 (58.04)	3.19	7.53	57.67 (49.56)
IIHR 13-15	21.67 (27.27)	0.59	11.24	94.74 (78.49)
Arka Karthik	25.00 (29.79)	0.29	3.55	91.92 (74.56)
Arka Apoorva	63.33 (52.89)	3.03	4.54	33.30 (35.02)
ArkaAjit	43.33 (41.09)	1.34	6.46	79.21 (64.62)
Arka Chaitra	75.00 (60.29)	1.40	7.94	82.43 (65.80)
IIHR 13-18	66.67 (55.34)	0.40	6.61	93.95 (76.18)
Swarna Mukti	93.33 (81.14)	1.52	7.26	79.02 (63.36)
ArkaSampoorna	68.89 (56.40)	0.60	7.01	91.45 (73.02)
VRPMR 10	61.67 (52.38)	0.80	11.07	92.74 (74.92)
PMR 53	55.00 (47.86)	1.36	7.20	81.09 (64.20)
IIHR 1-2	75.00 (60.24)	0.75	6.57	88.60 (70.34)
PC 531	58.33 (49.81)	0.71	6.15	88.42 (70.21)
PMR 37	98.33 (85.68)	1.58	6.33	75.02 (60.42)
IIHR 13-29	13.33 (17.59)	0.13	14.27	99.07 (85.45)
VRP 22	60.00 (50.77)	0.83	5.29	84.31 (66.79)
Oregon Sugar	45.00 (42.10)	0.47	7.84	93.96 (75.98)
Arka Tapas	56.67 (49.02)	0.45	5.27	91.42 (73.61)
IIHR 544	60.00 (51.00)	1.52	8.50	82.17 (65.26)
Azad P- 1	96.67 (81.37)	2.73	4.64	41.09 (39.76)
Arka Priya	61.67 (52.38)	0.88	5.49	84.06 (69.17)
Mean ± SEm	59.10 (52.22 ± 8.15)	1.28 ± 0.45	7.98	82.26 (67.42 ± 5.21)
CD at 1%	23.09	1.27	-	14.76
CV (%)	27.04	60.49	-	13.39

Table.6 Classification of different garden pea germplasm lines into different categories based on normal z-distribution

High temperature tolerant genotypes at seedling stage (IV quadrant)	Genotypes intermediate in response to high temperature stress at seedling stage (I and III quadrant)	High temperature susceptible genotypes at seedling stage (II quadrant)
ArkaUttam	Arka Chaitra	Arka Tapas
Arka Apoorva	Arka Priya	Arka Kartik
IIHR 544	Arka Pramod	IIHR 13-15
IIHR 13-1	ArkaAjit	IIHR 13-22
IIHR 680	ArkaSampoorna	IIHR 698
PMR 37	IIHR 13-18	IIHR 13-29
Swarna Mukti	IIHR 1-2	PMVAR 7
KTP 4	PMR 53	Oregon Sugar
VRPMR 11	VRPMR 10	PC 531
Azad Pea 1	VRP 22	PB29614 X 7-6
		Kashmir 13-1
		NDVP 24

Fig.1 Normal z-distribution of garden pea germplasm for heat tolerance or susceptibility



Screening for high temperature tolerance using TIR technique

The 48 h old seedlings of all the 32 germplasm lines were exposed to 30-38°C (3 h) induction temperature followed by 43°C (3h) lethal temperature to screen them for cellular tolerance to high temperature stress. The observations recorded at the end of recovery period showed significant differences in terms of percent seedling survival, recovery growth of seedlings and per cent reduction in growth over control (Table 4) for the different pea genotypes.

Among the genotypes IIHR 680 showed 100 percent seedling survival followed by genotype PMR 37 (98.33%), IIHR 13-1 (96.67%) and Swarna Mukti (93.33%). The least percent survival of 13.33% was observed in IIHR 13-29 followed by PB 29614 X 7-6 (20%), IIHR 13-15 (21.67%), Arka Karthik (25%) and Arka Pramod (38.33). Recovery growth of seedlings has been recorded highest in IIHR 13-1 (3.77 cm) followed by IIHR 680 (3.60 cm), KTP 4 (3.19 cm) and Arka Apoorva (3.03 cm), whereas, minimum values were observed in PB 29614 X 7-6 (0.11 cm), IIHR 13-29 (0.13 cm) and Arka Karthik (0.29 cm). Arka Apoorva showed the least per cent reduction in growth over control (33.30%) followed by KTP 4 (57.67%) and IIHR 13-1 (68.75).

The results obtained from screening experiment of different germplasms shows a huge variation in their thermotolerance, which may be due to different stress adaptive mechanisms. The results have revealed a range of 13.33 per cent seedling survival to 100 per cent seedling survival, the same has been observed in case of recovery growth of seedlings as well as the per cent reduction in growth. Similar kind of genetic variability for cellular tolerance has been reported by several researchers (Srikanthbabu *et al.,* 2002: Bharani, 2014; Chaukhande *et al.,* 2017; Sujatha *et al.,* 2018).

Assessment of garden pea lines for their reaction to high temperature stress using z-distribution analysis

Based on percentage seedling survived and percentage reduction in growth obtained from the screening results, z-score was calculated for all the 32 garden pea germplasm lines (Table 5), the genetic variability of 32 pea genotypes to heat tolerance was assessed by plotting their z-score on normal z-distribution graph (Figure 1). Genotypes were classified in three different categories, *viz*., high temperature tolerant (IV quadrant), moderately susceptible (I and III quadrant) and susceptible(II quadrant) based on z-analysis. The normal Z distribution revealed that the genotypes: ArkaUttam, Arka Apoorva, IIHR 544, IIHR 13-1, IIHR 680, PMR 37, Swarna Mukti, KTP 4, VPPMR 11 are tolerant to high temperature whereas Arka Chaitra, Arka Priya, Arka Pramod, ArkaAjit, ArkaSampoorna, IIHR 13-18, IIHR 1-2, PMR 53, VRPMR 10 and VRP 22 are moderately susceptible. Arka Tapas, Arka Karthik, IIHR 13-15, IIHR 13-22, IIHR 698, IIHR 13-29, PMVAR 7, PC 531, PB29614 X 7-6, Kashmir 13-1 and NDVP 24 were categorized as susceptible to high temperature (Table 6).

The normal Z-distribution technique, used for classification of genotypes into different groups based on parameters such as percentage seedling survived and percentage reduction in growth has been reported as promising by other researchers also, such as Srikanthbabu *et al.,* (2002) (garden pea), Chaukhande *et al.,* (2017) (French bean), etc.

The standardization studies using 48 h old Azad P-1 seedlings revealed that optimum lethal and induction temperature were 43°C for 3 hours and 30-38°C for 3 hours,

respectively. Screening of 32 garden pea germplasm lines using TIR technique resulted in identification of 10 tolerant lines (ArkaUttam, Arka Apoorva, IIHR 544, IIHR 13-1, IIHR 680, PMR 37, Swarna Mukti, KTP 4, and VRPMR 11) by the help of normal Z-distribution.

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